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FORM REGULATION IN CERIANTHUS.

II. THE EFFECT OF POSITION, SIZE AND OTHER FACTORS UPON REGENERATION.

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In the preceding paper (BIOL. BULL., Vol. V., No. 5, 1903), the course of regeneration in cylindrical pieces from the middle region of the body was described, since such pieces afford a typical result and serve as a basis for comparative study. It is desired in the present paper to call attention to certain conditions which influence the result, either as regards time or quantity.

The principal features in the regeneration of *Cerianthus* may be reviewed as follows: the collapse of the piece after section and the infolding of the ends; the closure of the ends by new tissue and the gradual distension of the piece and the increase in the area of the new tissue at the ends in consequence of the accumulation of water in the enteron, probably by diffusion through the body-wall; the reduction and disappearance of the muscular layer and pigment at both ends; the regeneration of mesenteries; the outgrowth from the tentacular ridge of a marginal tentacle over each intermesenterial chamber; the formation of the mouth in the directive radius; the appearance of the labial tentacles in a circle upon the disc; the outgrowth of new tissue at the aboral end of the piece.

Since each of these processes is gradual it is impossible to determine with exactness the time of its beginning; moreover, the various processes overlap and are connected in such a manner that it is difficult to separate distinct stages except arbitrarily. For these reasons the comparison of different pieces with a view to determining the conditions which effect regeneration can best be accomplished by the examination of these pieces at stated times, rather than by noting the time at which a given piece arrives at a particular stage. The former method not only allows direct comparison of the pieces, and thus often renders the detection of slight differences less difficult, but it obviates the necessity for

almost continuous observation and the accompanying manipulation necessary to examination, which is a source of irritation to the regenerating pieces and may often effect the result by causing rupture of new tissue or other injuries.

In general then the method pursued in the experiments was that of examining at intervals pieces to be compared and noting the condition of each. Owing to the number of points to be observed and the necessity for indicating slight differences any arrangement of the results in tables is unsatisfactory: they are given, therefore, in much the same manner as first recorded. The series of experiments described are selected from a large number but the results were remarkably uniform in all cases. In the description of the stages only the most salient features of the regeneration are mentioned in most instances. In all cases, however, unless definite statement is made to the contrary, regeneration proceeded in the typical manner.

I. DESCRIPTION OF EXPERIMENTS.

SERIES 22.¹

September 24, 1902. The oral end, including the œsophageal region, was removed from twenty-three large specimens of *C. solitarius* and the remaining portion of the body was divided by a transverse cut as nearly as possible into two equal pieces (Fig. 1), oral halves being designated A, aboral B. All of the pieces A were placed in one aquarium, all of B in another.

September 27: Three days after section:

A. Most of the pieces are still collapsed, but in a few the ends are closed and a slight distension with water is evident.

B. All still collapsed.

September 28: Four days after section:

A. All the pieces are more or less distended with water: three pieces show the tentacular ridge and the first traces of marginal tentacles.

B. Three pieces are closed and somewhat distended, the remainder still collapsed.

September 29: Five days after section:

¹ The series retain the numbers given them in my notes.

A. Distinct marginal tentacles are present on eight pieces ; the remainder all distended and with tentacular ridge.

B. All closed and more or less distended ; in a few distension is just beginning ; none with distinct tentacles.

September 30 : Six days after section :

A. All with distinct marginal tentacles from 0.5–1.0 mm. long.

B. The pieces which were the first to close and become distended show traces of marginal tentacle buds ; all pieces distended with water.

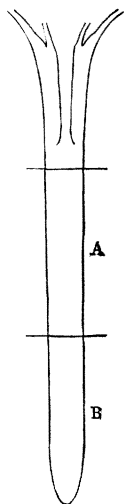


FIG. 1.

October 1 : Seven days after section :

A. All with marginal tentacles 1.0–2.0 mm. long.

B. Traces of marginal tentacles on all pieces except those which were the last to close.

This series was not kept under observation for the later stages. As regards the earlier stages, however, it shows clearly that the aboral pieces regenerate somewhat less rapidly than the oral pieces, although the latter are cut at both ends, the former at only one end. The difference between the two sets of pieces is universal, not even the most advanced pieces in the set B showing as rapid regeneration as the least advanced of A. In general the differences between pieces of the same set are slight.

SERIES 45.

November 7, 1902. Tentacles and disc were removed from four specimens and the remaining portion of the body was cut into four pieces, A, B, C, D, as nearly equal as possible (Fig. 2). All of the pieces A were placed in one aquarium, all of B in another, etc. The pieces A contained a part of the œsophagus.

November 9 : Two days after section :

A. Ends closed and piece distended ; as in other similar cases the cut oral margin of the œsophagus has united with the oral margin of the body-wall so that the pieces possess a well-developed mouth-opening.

B, C, D. All collapsed.

November 10 : Three days after section :

A. Marginal tentacular ridge appearing.

B. One piece beginning to fill with water ; others collapsed.

C. One piece beginning to fill ; others collapsed.

D. All collapsed.

November 12 : Five days after section :

A. Marginal tentacular ridge distinct, with fading pigment.

B. All distended ; new tissue at ends visible ; tentacular ridge forming.

C. One piece distended ; new tissue at ends visible ; two pieces partly filled but not sufficiently to show the new tissue at the ends ; one piece still collapsed.

D. All still collapsed.

November 15 : Eight days after section :

A. Marginal tentacles just appearing in all.

B. One piece with marginal tentacles just appearing ; three pieces distended ; new tissue at ends visible ; tentacular ridge distinct, unpigmented.

C. Two pieces distended ; new tissue at ends visible ; tentacular ridge forming ; one piece still collapsed ; one piece collapsed and completely enclosed in slime which was removed.¹

D. All still collapsed.

November 20 : Thirteen days after section :

A. Marginal tentacles in all 1 mm. in length.

B. In one piece marginal tentacles 1 mm., in others about 0.5 mm.

C. All distended ; tentacular ridge unpigmented, marginal tentacles just appearing.

D. One piece beginning to fill, others collapsed.

November 25 : Eighteen days after section :

A. Marginal tentacles 2-2.5 mm. showing faint traces of pigmented bands in two pieces, in other two unpigmented ; labial tentacles 0.5 mm. At aboral end new outgrowth 2 mm.

B. Marginal tentacles 1-2 mm., some differences in length appearing in individual pieces, unpigmented ; a few labial tentacles

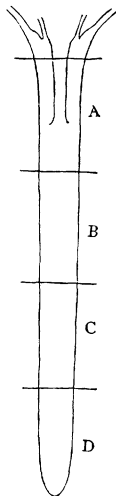


FIG. 2.

¹ The complete enclosure of pieces in slime, as in a cyst, often occurs when they remain collapsed for more than four or five days. The slime being secreted all over the ectoderm unites at the infolded ends and forms a complete cyst from which the piece is unable to emerge after a few days.

just appearing in each piece. At aboral end new outgrowth about 2 mm.

C. In two pieces marginal tentacles 0.5–1.0 mm., in other two slightly less advanced; in all unpigmented: none with labial tentacles. At aboral end no distinct outgrowth of new tissue.

D. One piece partly filled, others collapsed.

December 2: Twenty-five days after section:

A. Marginal tentacles about 5 mm., with distinct transverse pigment bands; labial tentacles 1–1.5 mm. Aboral end as before.

B. Marginal tentacles 3.5–4 mm., pigment bands visible but lighter than in A; labial tentacles 0.5–1 mm. Aboral end as before.

C. Marginal tentacles mostly 3 mm., a few in two pieces 4 mm., all unpigmented; labial tentacles just appearing. At aboral end no distinct outgrowth of new tissue.

D. One piece partly filled, but enclosed in slime which was removed; others still collapsed.

December 12: Thirty-five days after section:

A. Marginal tentacles 6–7 mm., pigment bands dark: labial tentacles about 2 mm. At aboral end outgrowth of new tissue 2–3 mm.

B. Marginal tentacles 5–6 mm.; pigment bands lighter than in A; labial tentacles 1–1.5 mm. At aboral end outgrowth of new tissue 2–3 mm.

C. Marginal tentacles 4–5 mm.; pigment bands visible, but slightly lighter than in B; labial tentacles about 1 mm. At aboral end outgrowth of new tissue 1 mm.

D. One piece closed and partly filled as before, but no traces of tentacular ridge. Three pieces still collapsed and enclosed in slime which was removed.

The series as a whole was concluded at this time, since the only further changes in A, B and C consist of a slight increase in length of the tentacles and the pigmentation. The pieces D, however, which had not as yet shown any traces of regenerating tentacles were kept under observation until January 21, 1903. Up to this time only the one piece which had become partly filled showed any signs of regeneration, the others remaining completely collapsed and surrounded by slime, which was removed

from time to time in order to permit distension to occur if there were any tendency. The changes during this time in the one piece which was closed and partly filled are of considerable interest. At one side of the closed oral end of the piece a few minute outgrowths 0.2–0.5 mm. in length made their appearance. They resembled marginal tentacles and were situated where these organs should appear, but there were only a few of them close together on one side and no others appeared. Fig. 3 shows the piece as it appeared January 21.

The new tissue closing the end is indicated by the stippling. At one side are six small outgrowths resembling tentacles, but no traces of any others can be found at any point of the circumference. At the



FIG. 3.

conclusion of the experiment the piece was opened and it was found that a few of the longest mesenteries extended into the piece in the radius in which the outgrowths appeared. This region is then without doubt the region of the directive mesenteries, and the mesenteries present are simply the longest mesenteries of the body which lie to the right and left of the short directives and extend nearly to the aboral end. The small outgrowths correspond in position with the spaces between these mesenteries and there can be little doubt that they represent marginal tentacles. No other mesenteries are present in the piece, none having regenerated. It becomes evident from the history of this piece that the presence of mesenteries is necessary for the regeneration of marginal tentacles. In pieces from regions nearer the oral end mesenteries are regenerated, but in this piece no trace of regenerated mesenteries could be found, and tentacles have begun to regenerate only in the spaces between such of the old mesenteries as extended into the piece.

The series as a whole affords several results of importance. As in the preceding series, the decreasing rapidity of regeneration with increasing distance from the oral end of the body is clearly shown. The pieces A regenerate more rapidly than B, B more rapidly than C, and finally D, the aboral pieces, are capable of only a slight degree of regeneration or of none at all, the difference between the one piece which regenerated a few tentacles and

the others probably being due to the fact that the cut separating C from D in the one case was slightly more oral than in the other three.

The differences in the rapidity and the amount of regeneration are best shown at the oral ends of regenerating pieces, for it is difficult to determine with exactness the amount of actual new tissue at the aboral ends of the pieces, since the line of demarcation between the unpigmented tip and the normally pigmented regions oral to it is not at all sharp, extending in many cases over two to three millimeters. As regards the aboral ends the pieces A and B showed little difference, but regeneration at the aboral end of C was in all cases distinctly less than in A and B.

In general the series seems to indicate that not only is regeneration less rapid with increasing distance from the oral end, but that there is a corresponding difference in the amount of regeneration. In the pieces A, B and C the differences are comparatively slight, though without doubt present as can be seen by comparing the data for these pieces thirty-five days after section. When the pieces D are taken into consideration, however, the difference between these and all other pieces is marked, for in no case did these aboral ends show anything approaching complete regeneration. There is then, according to these results, a rapid decrease in regenerative power near the aboral end of the body, and apparently complete absence of this power in an aboral region representing approximately one fifth of the body-length. As will be shown below, much smaller pieces than this from other regions of the body are capable of complete regeneration; moreover, the size of the area within which regeneration does not occur differs according to conditions.

SERIES 54 AND 55.

December 15, 1902. The tentacles, disc and œsophageal region were removed from twenty large specimens; ten of the remaining pieces were then divided by a transverse cut into two pieces, A and B (Fig. 4), the cut being made near the aboral end so that the pieces A comprised the greater part of the body aboral to the œsophagus, while the pieces B represented the extreme aboral end, about one sixth of the body-length. These two sets of pieces constituted Series 54.

Each of the remaining ten pieces was also divided by a transverse cut into two pieces A and B, but in this case the cut was made near the oral end of the piece (Fig. 5), so that A repre-

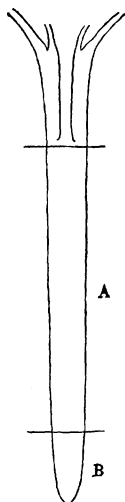


FIG. 4.

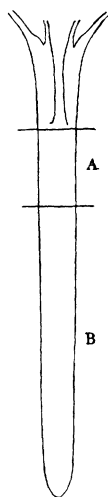


FIG. 5.

sents one sixth of the body-length from the region just aboral to the œsophagus, while B is the whole remaining portion of the body. These pieces constitute Series 55.

In this manner four sets of ten pieces each were obtained. The oral ends of pieces 54A and 55A represent approximately the same level in the body of the individuals from which they were taken, while their aboral ends lie at very different levels; moreover, the pieces 54A are about four times as long as 55A. The oral ends of the pieces 54B and 55B are at very different levels, while their aboral ends are the aboral ends of the parent bodies; 55B is about four times as long as 54B.

In 54A and 54B we have pieces differing widely in size and with oral ends at very different levels; the same is true of 55A and 55B, but the relations of size are reversed.

The comparative study of the regeneration of those four sets of pieces should afford data regarding the rapidity and amount of regeneration at different levels of the body and in pieces of different sizes.

December 19: Four days after section:

54A. All with closed ends and filling with water, but ends not yet expanded so as to show new tissue.

54B. All collapsed.

55A. All with closed ends and filling with water, but ends not yet expanded. Condition same as in 54A.

55B. Ends closed, but pieces contain less water than 54A and 55A.

December 22 : Seven days after section :

54A. All distended, ends expanding, new tissue visible ; tentacular ridge just appearing.

54B. All collapsed.

55A. Similar to 54A.

55B. All filling with water, but none distended sufficiently to expand the ends and show new tissue.

December 24 : Nine days after section :

54A. Tentacular ridge distinct, with fading pigment.

54B. All still collapsed.

55A. Similar to 54A.

55B. All distended, ends expanded and tentacular ridge just appearing.

December 26 : Eleven days after section :

54A. Marginal tentacles just appearing in some specimens as minute outgrowths from tentacular ridge, which is now unpigmented.

54B. All still collapsed.

55A. Similar to 54A.

55B. Tentacular ridge with fading pigment, but less distinct than in 54A ; no marginal tentacles.

December 28 : Thirteen days after section :

54A. Marginal tentacles 0.25–0.5 mm.

54B. All still collapsed.

55A. Similar to 54A.

55B. Tentacular ridge distinct, unpigmented in most cases ; in a few the earliest traces of marginal tentacles visible.

January 3, 1903 : Nineteen days after section :

54A. Marginal tentacles 2–3 mm.

54B. All still collapsed, much contracted, rounded in form.

55A. Similar to 54A.

55B. Marginal tentacles 1-1.5 mm.; in one specimen with rather unequal tentacles a few 3 mm.

January 11 : Twenty-seven days after section :

54A. Marginal tentacles 5-6 mm.; transverse bands of pigment distinct; labial tentacles 1-1.5 mm. At aboral ends no well-marked outgrowth of new tissue; ends slightly lighter in color at region of closure.

54B. All still collapsed, much contracted, rounded in form.

55A. Oral ends similar to 54A. At aboral ends a distinct outgrowth of new tissue 2-3 mm.

55 B. Marginal tentacles 3-5 mm.; pigmentation of tentacles slightly less deep than in 54 A; labial tentacles just visible — 1 mm.

January 21 : thirty-seven days after section :

54 A. Marginal tentacles 7-8 mm.; labial tentacles 1-1.5 mm. No distinct outgrowth of new tissue at aboral ends.

54 B. Collapsed, rounded and still further reduced in size.

55 A. Marginal tentacles 6-8 mm.; labial tentacles 1-1.5 mm. At aboral ends distinct outgrowth of new tissue 3-5 mm. At this time the average length of the marginal tentacles in these pieces is somewhat less than in 54 A. In the latter cases there are fully as many specimens with tentacles 8 mm. in length as with tentacles 7 mm. In 55 A, however, only a few pieces, and these the largest, possess marginal tentacles 8 mm. in length; in nearly all the marginal tentacles are 6-7 mm. Moreover, the average length of the labial tentacles in 55 A is slightly less than in 54 A. These pieces are evidently falling behind the longer pieces.

55 B. Marginal tentacles 5-6 mm., somewhat less deeply pigmented than in 54 A; labial tentacles 1 mm.

At this time the regenerated structures had acquired their maximum size; afterward reduction in size, which always occurs in the pieces kept without food, began. For present purposes it is not necessary to follow the history of these pieces further.

Comparison of the data afforded brings to light a number of interesting results. Comparing the rapidity of regeneration in the different pieces, it is seen that the oral ends of pieces 54 A and 55 A, which represent approximately corresponding regions of

the parent body, regenerate with equal rapidity except at the end of the experiment, although pieces 54 A are about four times as long as pieces 55 A. The oral ends of pieces 55 B, which represent a region of the parent body further aboral than those of 54 A and 55 A, regenerate less rapidly than these, although the pieces are about equal in size to 54 A and four times as long as 55 A. And finally, the pieces 54 B, whose oral ends represent a region near the aboral end of the parent, do not regenerate at all.

As regards the aboral ends of the pieces only 54A and 55A need be considered, since no regeneration occurs at the aboral end of a piece when this represents the aboral end of the parent-body, as is the case in 54B and 55B. In 54A and 55A the difference in the rapidity and amount of regeneration at the aboral ends is marked; in 55A, where the aboral ends of the pieces represent a region oral to the middle region of the parent-body the aboral regeneration was much greater than in 54A, where the aboral ends represent a region near the aboral end of the parent-body, even though the pieces 54A were four times as long as 55A.

From all of these facts it is evident that the rapidity and amount of regeneration decrease as the cut surface, either oral or aboral, approaches the aboral end of the parent-body, and that the size of the piece has no marked influence, at least within the limits of size of the present experiment. That the size of the piece does, however, affect the final result in some degree is shown by the condition of pieces 54A and 55A at the end of the experiment 37 days after section; while no differences between the two sets were noted earlier it was found at this time that the smaller pieces 55A were falling slightly behind the larger 54A. Here then a slight influence of size is noticeable, though only in the later stages of the experiment. As will be shown later this result is confirmed by other cases. In pieces above a certain minimal size regeneration is not influenced by the size, except in the later stages.

SERIES 35.

October 20, 1902. In this case after removal of disc and tentacles a single specimen was cut into four pieces, A, B, C, D as shown in Fig. 6. The piece B was much smaller than the

others and masses of the mesenterial filaments protruded from each end, thus delaying the closure and normal regeneration; it is therefore omitted from the present consideration. The pieces A and C are nearly equal in length and are about two thirds the length of D.

October 22 : two days after section : All pieces still collapsed.

October 23 : three days after section:

A. Margin of œsophagus united with body-wall, aboral end closed and enteron partly filled with water.

C. and D. Both still collapsed.

October 24 : Four days after section :

A. Sufficiently distended with water to spread the inrolled margins and allow the œsophagus at the oral end and the new tissue closing the aboral end to become visible.

C. and D. Both still collapsed.

October 25 : Five days after section :

A. Distended with water : tentacular ridge visible and pigment disappearing from it.

C. Ends closed by new tissue ; distended.

D. Enteron partially filled with water ; distension not yet sufficient to separate the infolded oral margins and permit new tissue to become visible.

October 27 : Seven days after section :

A. Marginal tentacles just appearing on tentacular ridge.

C. Tentacular ridge distinct ; its pigment disappearing.

D. Distended with water ; new tissue closing oral end exposed by separation of cut margins in consequence of distension : tentacular ridge visible, with fading pigment.

October 29 : Nine days after section :

A. Marginal tentacles 1 mm.

C. Marginal tentacles just appearing on tentacular ridge.

D. Tentacular ridge distinct, without pigment ; no tentacles visible.

October 31 : Eleven days after section :

A. Marginal tentacles 2 mm. At aboral end new tissue growing out in a small point 1.5 mm.

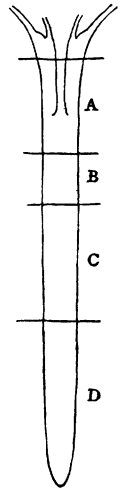


FIG. 6.

November 6 : Seventeen days after section :

A. Marginal tentacles 5 mm.; labial tentacles 0.5–1 mm. At aboral end outgrowth of new tissue 2 mm.

C. Marginal tentacles 3–4 mm.; labial tentacles 0.5. At aboral end of outgrowth of new tissue 1 mm.

D. Marginal tentacles 1–2 mm.; labial tentacles not yet visible.

November 12 : Twenty-three days after section :

A. Marginal tentacles 10 mm.; distinctly marked with the characteristic transverse bands ; labial tentacles 2–3 mm. At aboral end margins of old body-wall are becoming involved in the growth and losing pigment ; the unpigmented area, including outgrowths, is about 3 mm. in length from aboral end.

C. Marginal tentacles 6–7 mm.; transverse pigment bands visible but not dark as in A ; labial tentacles 2 mm. At aboral end new outgrowth 1.5 mm.

D. Marginal tentacles 5–6 mm. still unpigmented ; labial tentacles 1 mm.

November 20 : Thirty-one days after section :

A. Marginal tentacles 12–13 mm.; pigment bands dark and distinct ; labial tentacles about 3 mm. Aboral end as before.

C. Marginal tentacles 8–9 mm.; pigment bands distinct but less dark than in A ; labial tentacles 2–2.5 mm. At aboral end unpigmented area 2–3 mm. in length.

D. Marginal tentacles 6–8 mm.; pigment bands visible but less dark than in C ; labial tentacles 2 mm.

December 2 : Forty-three days after section :

A. Marginal tentacles 12–13 mm.; labial tentacles 3–4 mm. At aboral end the new outgrowth is becoming pigmented.

C. Marginal tentacles 10 mm.; pigmentation of tentacles scarcely distinguishable from that of A ; labial tentacles 3 mm. At aboral end the unpigmented area about 3 mm.

D. Marginal tentacles 12–13 mm.; pigmentation slightly less dark than that of A ; labial tentacles 3–4 mm.

At this time regeneration is essentially complete in the pieces ; no further increase in the length of tentacles, or of the new growth at the aboral end occurs. The marginal tentacles of C and D are still slightly lighter in color than those of A, and the pigment has not yet extended over the aboral outgrowth in C as far as in A, but these slight differences are later eliminated.

Examination of the data shows that at all stages except the final A is more advanced in regeneration than C, and C more advanced than D.

It will be noted also that the regenerated parts of piece A did not increase in size after 31 days, with the exception of the labial tentacles which showed a slight increase between 31 and 43 days. In the piece C a slight increase in the length of all tentacles occurred between 31 and 43 days. In the piece D, however, there was a marked growth during this time. In other words the piece A completed its regeneration first, then the piece C, and last of all the piece D.

Throughout this series then there is a distinct relation between the rapidity of regeneration and the position of the pieces in the parent-body, the rapidity of regeneration decreasing with increasing distance from the oral end.

One other point requires consideration: the regenerated tentacles of the piece D finally attain the same length as those of piece A. This would appear at first glance to contradict the results obtained from other series of experiments where not only the rapidity but the amount of regeneration diminishes toward the aboral end. Comparing A and C, two pieces about equal in size, we find that the amount of oral regeneration in A is greater than in C, as might be expected from comparison with other series, since C represents a region farther from the oral end of the parent-body than A. The piece D, still nearer the aboral end of the parent-body, but much longer than A and C, while regenerating more slowly than either of these finally equals A in the amount of regeneration. Apparently in this case the influence of size has counterbalanced the influence of position. If piece D was of the same size as A and C the amount of oral regeneration would undoubtedly be less than in those pieces, but since it is much larger, *i. e.*, contains much more available material, regeneration continues for a somewhat longer time (note the increase in size of tentacles in D between 31 and 43 days) and the regenerated organs finally, though after a longer time, reach a condition similar to that in A. In this case, as in Series 54 and 55, the influence of size is slight and appears only in the latest stages of regeneration.

SERIES 56.

December 15, 1902. Disc, tentacles and œsophageal region were removed from ten large specimens by a transverse cut aboral to end of œsophagus. The aboral piece was then cut into two pieces, A and B, of equal length (Fig. 7) which were kept for comparison.

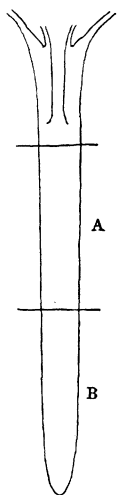


FIG. 7.

December 19: four days after section:

A. Nine pieces with ends closed; a few distended, the others partly filled with water; one piece still collapsed.

B. All still collapsed.

December 22; seven days after section:

A. All distended; ends well expanded, showing new tissues in a few pieces the tentacular ridge is just appearing.

B. All still collapsed.

December 26: Eleven days after section:

A. Tentacular ridge distinct, with faded pigment; in a few pieces the first traces of marginal tentacles distinct.

B. Filling with water but not distended; new tissue at oral end not visible.

December 28: Three days after section:

A. Marginal tentacles 0.5 mm.

B. Four pieces fairly well filled with water; tentacular ridge just visible; six pieces collapsed or only partly filled; tentacular ridge not visible.

January 3, 1903: Seventeen days after section:

A. Marginal tentacles 2-3 mm.

B. One piece distended; marginal tentacles 2 mm. Nine pieces partly or completely collapsed; no tentacular ridge or tentacles visible.

Circumstances necessitated the conclusion of the series at this time, so that it was impossible to determine whether the nine pieces of B would ever have regenerated. The series affords, however, some interesting results. As in all other series regeneration is much less rapid in the aboral pieces; in only one case did the aboral pieces regenerate tentacles before the conclusion

of the experiment. Examination of the data shows that the pieces B were all filling with water eleven days after section ; that two days later all but four were collapsed, and that, finally, seventeen days after section, only one piece was filled with water. These changes are undoubtedly due to the fact that the growth of new tissue at the ends of these pieces failed to keep pace with the pressure of water in the enteron, and so rupture occurred as soon as the pieces reached a certain point. In only one case did the new tissue remain intact, viz., the case in which tentacles appeared.

As is evident from a comparison with other series, viz., series 22, 35, and 45, regeneration was found to occur in other cases in pieces representing about the aboral third of the body, though pieces representing the aboral fifth (series 45) or less did not regenerate. Why then did regeneration fail to occur in the aboral pieces of the present series? The difference is undoubtedly to be accounted for by the low temperature of the water. This series was begun in December and continued into January. The temperature of the water was very much lower at this time than during the autumn, and several other series begun on the same date showed similar results. In other words, the area at the aboral end which is incapable of regeneration increases as the temperature becomes lower, and in the present series includes more than the aboral third of the body. This point will be discussed in a following section where the influence of temperature is considered.

(To be Continued.)